World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2025
Volume: 4; Issue: 2
Page: 619-626

ANTIMICROBIAL AND CYTOTOXIC PROPERTIES OF *PHYLLANTHUS NIRURI:* A PHYTOCHEMICAL APPROACH

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Article Received: 26 February 2025 | | Article Revised: 15 March 2025 | | Article Accepted: 06 April 2025

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DOI: https://doi.org/10.5281/zenodo.15202340

How to cite this Article: Parvathy Prasad, Gadha Vijayan, Gowri Gopan, Henna Jahas, Panchami Sankar (2025). ANTIMICROBIAL AND CYTOTOXIC PROPERTIES OF *PHYLLANTHUS NIRURI*: A PHYTOCHEMICAL APPROACH. World Journal of Pharmaceutical Science and Research, 4(2), 619-626. https://doi.org/10.5281/zenodo.15202340

ABSTRACT

Phyllanthus niruri (P.niruri) has garnered attention for its potential medicinal and therapeutic properties attributed to its rich phytochemical composition. *P.niruri*, possess diverse applications across multiple domains. The qualitative analysis of hydro- ethanolic extract of *P.niruri* showed the presence for flavonoids and was found to be 689.3µg/ml. *P.niruri* showed no anti-bacterial activity against *S.typhi* and *E.coli*. The anti-fungal activity of *P.niruri* showed zone of inhibition at 50µg/ml against *C.albicans* and no inhibitory activity was found against *A.niger*. The cytotoxic activity of *P.niruri* was conducted using HepG2 cell lines and showed a decrease in percentage of inhibition.

KEYWORDS: *Phyllanthus niruri*, Phyochemical, Anti-bacterial, Anti-fungal, Cytotoxic activity.

INTRODUCTION

Phyllanthus niruri (P.niruri), also known as stone breaker, is a tropical perennial plant found across diverse habitats, from coastal areas to highlands. It belongs to the family Euphorbiaceae and grows as an erect, slender herb, 10 - 50 cm tall, with small elliptical leaves, tiny and red-flushed flowers. The plant propagates through seeds, which are dispersed by water or animals, and is commonly found in gardens, roadsides, and agricultural lands.[1]

P.niruri is widely used in traditional medicine across Asia, America, and the Caribbean for treating a range of ailments, including jaundice, diabetes, skin diseases, gall bladder stones, hypertension, and liver conditions. It is also used in cosmetics and for preparing hair oils, dyes, and tooth powders.[1]

The plant is known for its pharmacological properties, including anti-inflammatory, anti-tumor, anti-oxidant, anti-viral, anti-malarial, anti-ulcer, analgesic, hepatoprotective, and anti-cancer activities. These effects are attributed to its rich content of phytochemicals, such as lignans, alkaloids, tannins, flavonoids, and triterpenes, which helps to protect against pathogens and support human health.[2,3]

The anti-microbial activity of *P.niruri* has been well-documented, showing effectiveness against bacteria like *Streptococcus mutans, E. coli*.[4] Studies indicate that extracts of this plant inhibit the growth of common gastrointestinal and skin infection-causing bacteria.[5,6] However, its anti-fungal properties are limited, with little inhibitory effect on *Candida albicans* and *Aspergillus niger*.[7]

P. nururi is used by the people from time immemorial as anti-cancerous agent. A study carried out to evaluate the anti-tumor activity of hydro-alcoholic extract of the whole plant showed significant reduction in tumour incidence, tumour yield, tumour burden and cumulative number of papillomas as compared to carcinogen-treated controls.[6] Corilagin is the major active anti-tumor composition in *P.niruri* and has great inhibiting effect on HCC cells.[8] When combined with Curcuma longa, *P.niruri* demonstrated the anti- proliferative effect on MDAMB-231 cell lines.[9] Phyllanthin (the major constituent in the decoction of *P.niruri*) treating hepatic-related diseases by inhibiting HepG2 cell proliferation, inducing apoptosis in HepG2 via caspase-3-dependent cell death mechanism.[10]

METHODOLOGY

Sample Preparation

Healthy and mature *P.niruri* plants were collected from a local garden in Thiruvananthapuram, washed with distilled water, and dried at room temperature (37°C). The aerial parts were weighed, placed in filter paper, and loaded into the thimble of a Soxhlet apparatus for extraction using hydroethanol (70% ethanol and 30% water) as the solvent. The process involved continuous refluxing of the solvent through the plant material at 60°C, with water circulation in the condenser to maintain a low temperature.

Screening of phytochemicals

Qualitative analysis

Test for Flavonoids: The extract (1 mg/ml) was prepared in hydroethanol (HET, 70:30). To this, 2 mL of 2% NaOH solution was added. A positive result was indicated by the formation of an intense yellow colour that became colourless upon the addition of dilute acid.

Test for Alkaloids: The extract (1 mg/ml) in HET (70:30) was treated with 1 - 2 drops of Wagner's reagent along the test tube walls. A positive result was indicated by the formation of a brown or red precipitate.

Test for Saponins: The extract (1 mg/ml) in HET (70:30) was mixed with 1 mL of double-distilled water and shaken thoroughly. A stable foam formation indicated positive result.

Quantitative analysis - Estimation of Flavonoid: Quercetin standards (200 - 1000 µg/ml) and blank (1 mL solvent) were prepared. To each, 0.3 mL of 5% sodium nitrate was added, followed by the addition of 1 mL of 10% aluminum chloride and after 5 minutes, 2 mL of 1 M sodium hydroxide was added. The mixtures were incubated at room temperature for 10 minutes, diluted to 10 mL with water, and absorbance was measured at 510 nm. A calibration curve was plotted using Quercetin standards, and the sample's flavonoid content was determined using the curve.

Determination of Antibacterial and Antifungal activities Media Preparation: Nutrient agar (NA) was prepared by dissolving 1.3 g of nutrient broth (NB) and 1.5 g of agar in 100 mL of distilled water (DW). Potato dextrose agar (PDA) was prepared by mixing 0.13 g of PDA in 100 mL of DW. Both media were autoclaved at 121°C and 15 lbs pressure for 15 minutes, poured into sterile petri dishes, and allowed to solidify.

Preparation of Nutrient Broth for Bacterial Culture: For bacterial culture, 0.13 g of NB was dissolved in 100 mL of DW, and 5 ml was dispensed into two test tubes. After autoclaving at 121°C for 15 minutes, bacterial strains *E. coli* and *S. typhi* were inoculated and incubated at 37°C for 24 hours.

Preparation of Potato Dextrose Broth for Fungal Culture: For fungal culture, 0.39 g of potato dextrose was dissolved in 100 mL of DW, and 5 mL was dispensed into two test tubes. After autoclaving, fungal strains such as *Candida albicans* and *Aspergillus niger* were inoculated and incubated at 37°C for 24 hours.

Stock Preparation: 1 mg/mL stock solution of *P.niruri* extract was prepared in HET (70:30). Working concentrations of 200µg/mL, 50µg/mL and 5µg/ml were prepared.

Agar Well Diffusion Method: Nutrient agar plates were swabbed with *E. coli* and *S. typhi*, while PDA plates were swabbed with *A. niger* and *C. albicans*. Wells were loaded with ciprofloxacin (positive control), HET (negative control), and *P.niruri* extract at different concentrations such as 200 µg/mL, 50 µg/mL, and 5 µg/mL. Plates were incubated at 37°C for 24 hours, and zone of inhibition were measured.

Cell Cytotoxicity Assay: HEPG2 cells (0.1×10^6 cells/well) were seeded into 24-well plates containing 500µL of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), antibiotics (500 µL), and gentamycin (400 µL). Cells were incubated in a carbondioxide (CO_2) incubator at 37°C for 24 hours, for cell attachment, followed by incubation to achieve confluency. Cells were treated with various concentrations of *P.niruri* extract along with control and incubated for an additional 24 hours in DMEM containing 5% FBS. Following treatment, cells were washed with 500 µL phosphate-buffered saline (PBS) and incubated with 300 µL of MTT solution in 500 µl PBS for 2 - 4 hours. The resultant purple formazan formed in live cells was solubilized with 200 - 300 µL of dimethyl sulfoxide (DMSO) for 15 - 30 minutes. Absorbance was measured at 570 nm using spectrophotometer.

RESULTS

Phytochemical screening of *P.niruri*

In phytochemical screening, qualitative analysis results showed that flavonoid was positive compared to alkaloids & saponins.

Qualitative analysis of phytochemicals in *P.niruri*

The phytochemical content of *P.niruri* is depicted in the figure below (Fig 1). Test tube A showed absence of reddish-brown precipitate which indicated the absence of alkaloids. Test tube B showed yellow color solution, which turned colorless while adding dilute acid, confirmed the presence of flavonoids. Test tube C showed no stable foam which showed absence of saponins.

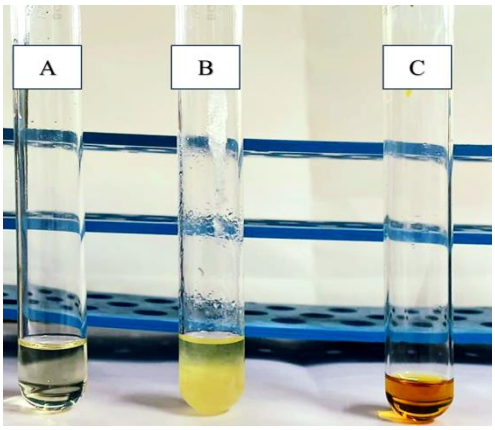


Figure 1: Phytochemical screening for the identification of (A) flavonoids (B) alkaloids and (C) saponins.

Quantitative analysis of flavonoids at different concentrations

The quantitative analysis of flavonoids at different concentrations was performed, with hydroethanolic extract of *P.niruri* showing the highest absorbance value of 0.880. The absorbance values for quercetin, used as standard at different concentrations - 5µg/mL, 50µg/mL, and 200µg/mL, were recorded as 0.212, 0.370, and 0.416, respectively (Table 1). The total flavonoid content of the sample, calculated using the quercetin standard curve equation = 0.0009X+0.2596 at an absorbance of 0.880, was determined to be 689.3µg/ml (Fig2). These results highlight the significant flavonoid content in *P.niruri* extract compared to standard.

Concentration (µg/ml)	Absorbance (570nm)
5	0.212
50	0.37
200	0.416
Sample	0.88

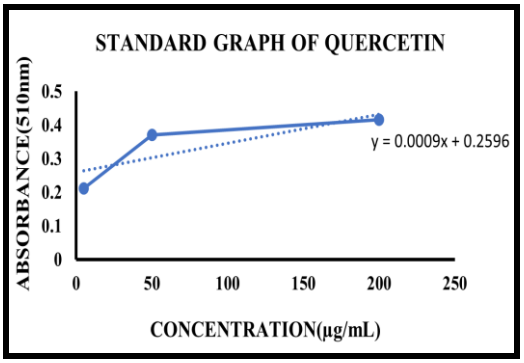
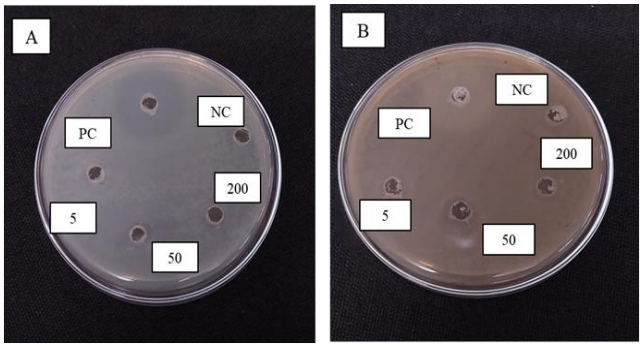


Table 1 & Figure 2: Quantitative analysis of flavonoid at different concentrations of quercetin as standard 5µg/ml, 50µg/ml, 200µg/ml and sample and Standard graph of Quercetin.

Anti-microbial analysis

Agar well diffusion of Hydroethanol Extract of *Phyllanthus Niruri*

The figure given below is the agar plates which showed the anti-microbial activity of *P.niruri*. Different concentrations of 5µg/ml, 50µg/ml and 200µg/ml were used for analyzing the anti-microbial activity. No zone of inhibition was observed for the sample against *E. coli* and *S. typhi*, the diameter of positive control was measured as 26 and 35 mm respectively (Fig 3 and Table 2).

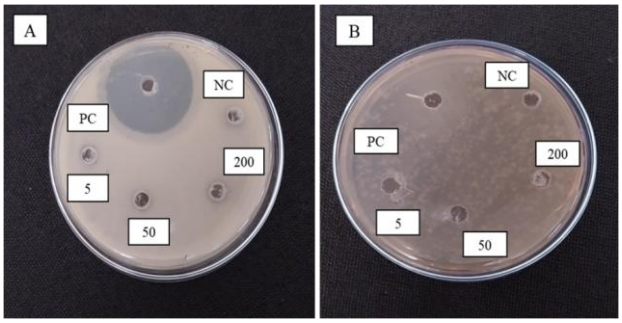


Concentration (µg/ml)	Zone of Inhibition (mm)	
	E. coli	*S. typhi*
PC (Gentamycin)	26	35
NC	-	-
5	-	-
50	-	12
200	-	-

Figure 3 & Table 2: Anti-bacterial analysis of hydroethanol extract of *Phyllanthus niruri.* Zone of inhibition of hydroethanolic extract of *P.niruri* A): - against *E. coli* and B): - against *S. typhi.*

Antifungal Analysis of Hydroethanol Extract of *Phyllanthus niruri*

The figure given below is the anti-fungal activity of *P.niruri* (Figure 4). Different concentrations of 5µg/ml, 50µg/ml and 200µg/ml were used for analyzing the anti-fungal activity. Gentamycin was used as positive control. *P.niruri* showed zone of inhibition at 50µg/ml against *C. albicans*, but *P.niruri* showed no zone of inhibition against *A. niger.*



Concentration (µg/ml)	Zone Of Inhibition (mm)	
	C.albicans	*A.niger*
PC (Gentamycin)	12	26
NC	-	-
5	-	-
50	-	-
200	-	-

Figure 4 & Table 3: Anti-fungal analysis of hydroethanol extract of *P.niruri* and Zone of inhibition of hydroethanoic extract against A):- *C. albicans* and B):- *A.niger*.

P.niruri - Cytotoxic activity on HepG2 cells

The cytotoxic activity of *P.niruri* was observed in HepG2 cells. The figure given below showed cytotoxic activity on HepG2 cells (picture under inverted microsope) and the same has been depicted in formazan crystal formation (Fig: 5 and Fig 6). The table: 4 given below showed that as the concentration of *P.niruri* increases, the percentage inhibition decreases.

Table 4: The Cell viability and Percentage Inhibition of Cells.

SI. No	Concentration	Absorbance				Cell Viability	% of Inhibition
		1	2	3	Mean		
1	Control	0.137	0.14	0.139	0.139	100	0
2	5	0.08	0.081	0.084	0.082	59	41.01
3	10	0.11	0.015	0.012	0.013	9.35	90.65
4	50	0.021	0.022	0.023	0.022	15.83	84.17
5	200	0.034	0.037	0.033	0.035	25.18	74.82
6	400	0.038	0.042	0.039	0.04	28.78	71.22
7	600	0.069	0.064	0.061	0.065	46.76	53.24
8	800	0.071	0.074	0.076	0.074	53.24	46.76

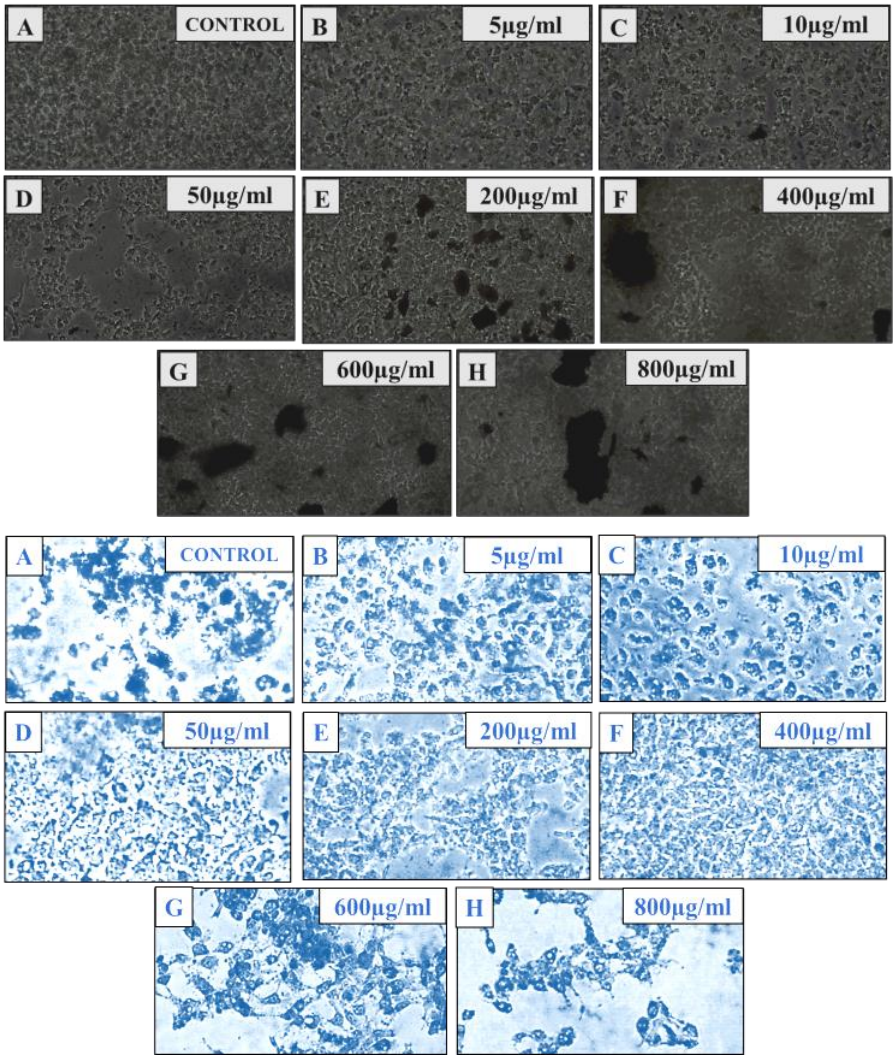


Figure 5 & Figure 6: : Phase contrast inverted microscope picture showing effect of *P.niruri in different sample concentrations (B-H) and control (A)* and Formazan crystal formation in different concentrations of *P.niruri* (B-H) and control (A).

DISCUSSION

The present study was conducted to evaluate the phytochemical, anti-microbial and cytotoxic effect of hydro-ethanolic extract of *P.niruri*. The hydo-ethanolic extract of *P.niruri*. showed the presence for flavonoids and absence of alkaloids and saponins. Quantification of flavonoid was further validated and found to be 689.3µg/ml. Previous study revealed that water along with hydroalcoholic extract of *P.niruri* showed the presence for flavonoid and absence of alkaloids and saponins.[11] In our study the anti-bacterial activity was analysed using agar well diffusion at different concentrations of hydroethanolic extract of *P.niruri* extract. No zone of inhibition was observed against *E.coli* and *S. typhi*.

The study on dimethyl sulphoxide extract of *P.niruri* showed no zone of inhibition against *E.coli.*[12] Aqueous leaf extract of *P.niruri* showed no zone of inhibition for *S.typhi.*[13] In our study the anti-fungal activity was done against *C. albicans* and *A. niger*. The study showed zone of inhibition only at 50µg/ml against *C. albicans* and no zone of inhibition observed at 5 and 200µg/ml. *P.niruri* did not show any zone of inhibition against *A. niger*. According to a previous study, *P.niruri* did not show any zone of inhibition against A. *niger* and *C. albicans.*[7] Further studies are required to elucidate the anti-fungal activity of *P.niruri against C. albicans & A. niger. P.niruri* showed effect on

HepG2 cells after 24 hours of incubation and found that as the concentration increased the cell viability increased and percentage of inhibition decreased. The hydroethanolic extract of *P.niruri* showed cytotoxic effect on HepG2 cell line. Previous research study showed that *P.niruri* has cytotoxic effect on both HepG2 and HT29 cell line.[14]

CONCLUSION

Experiments were conducted to evaluate the phytochemical activity of *P.niruri* and proved the presence of flavonoids and absence of alkaloids and saponins. The anti-bacterial activity was analyzed using agar well diffusion method and no zone of inhibition was observed against *E. coli* and *S. typhi.* The anti-fungal activity was also done by agar well diffusion and it showed zone of inhibition at 50 µg/ml against *C. albicans* and no zone of inhibition was observed for *A. niger*. *P.niruri* showed cytotoxic effect on HepG2 cells.

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